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A).

1 MALWMRLPL LALLALWGPD PAAAFVNGHL CGSHLVEALY LVCGERGFFY  
51 TPKTRREAED LQVGQVELGG GPGAGSLQPL ALEGLGKRG IVEQCCTSIC  
101 SLYQLENYCN

B).

A-chain

B-chain

1 GIVEQCCTSI CSLYQLENYC NFVNQHLGSH HLVEALYLVC GERGFFYTPK T

C).

1TRZ:A

1 GIVEQCCTSI CSLYQLENYC N  
HHHHTTS HHHHGGG

1TRZ:B

1 FVNQHLGSH LVEALYLVC GERGFFYTPKT  
S HHH HHHHHHHHHG GG

1TRZ:C

1 GIVEQCCTSI CSLYQLENYC N  
HHHHSSS HHHHGGG

1TRZ:D

1 FVNQHLGSH LVEALYLVC GERGFFYTPKT  
HHHHHHH HHHHHHHHHG GG S

Figure 1

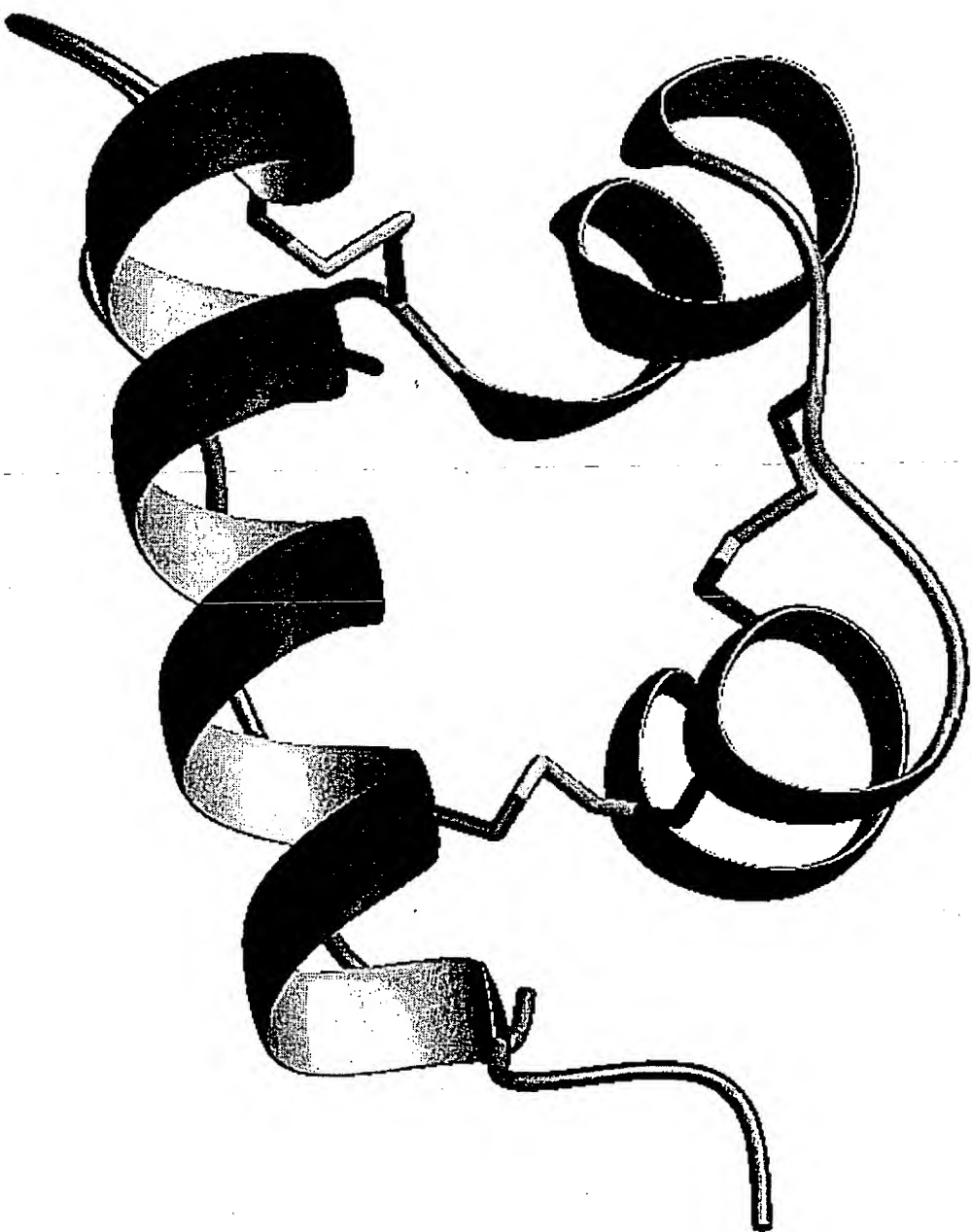
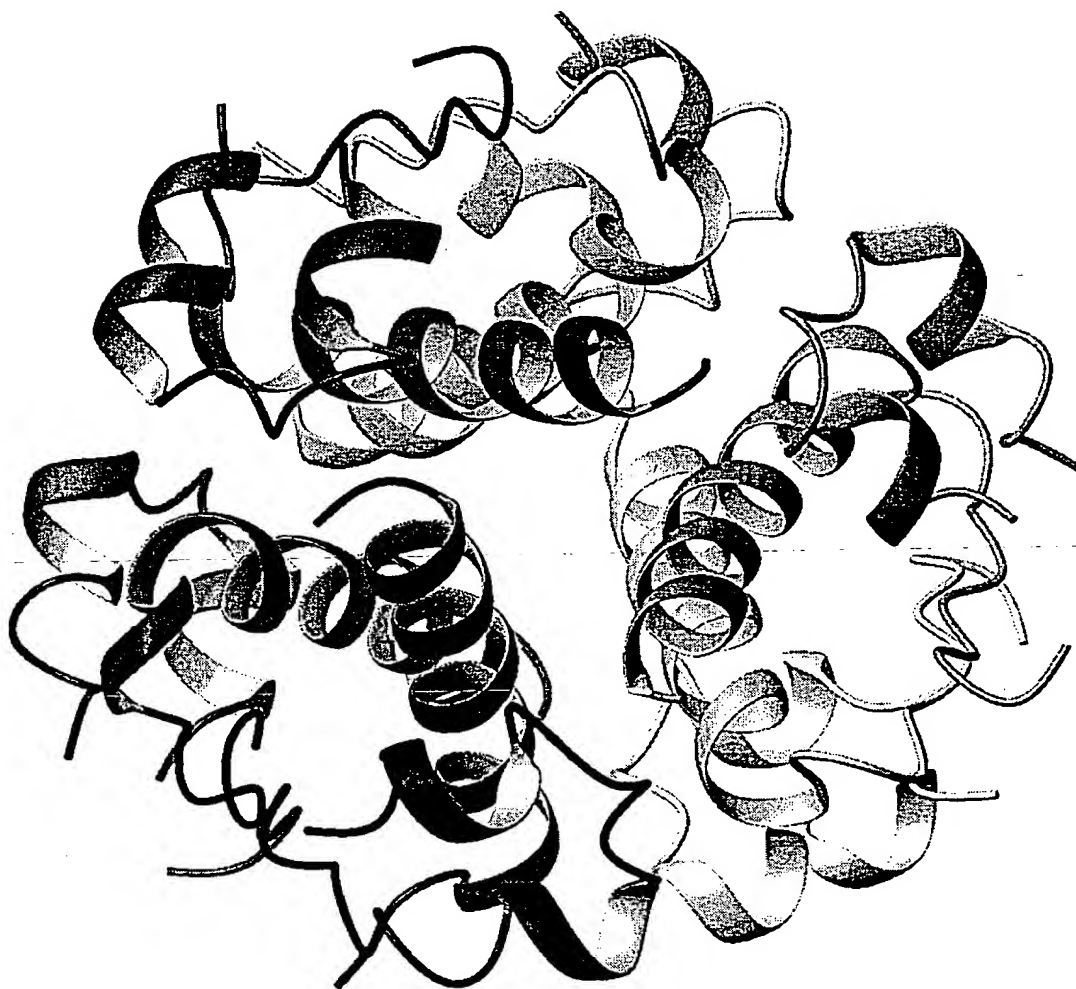


FIGURE 2A



**FIGURE 2B**

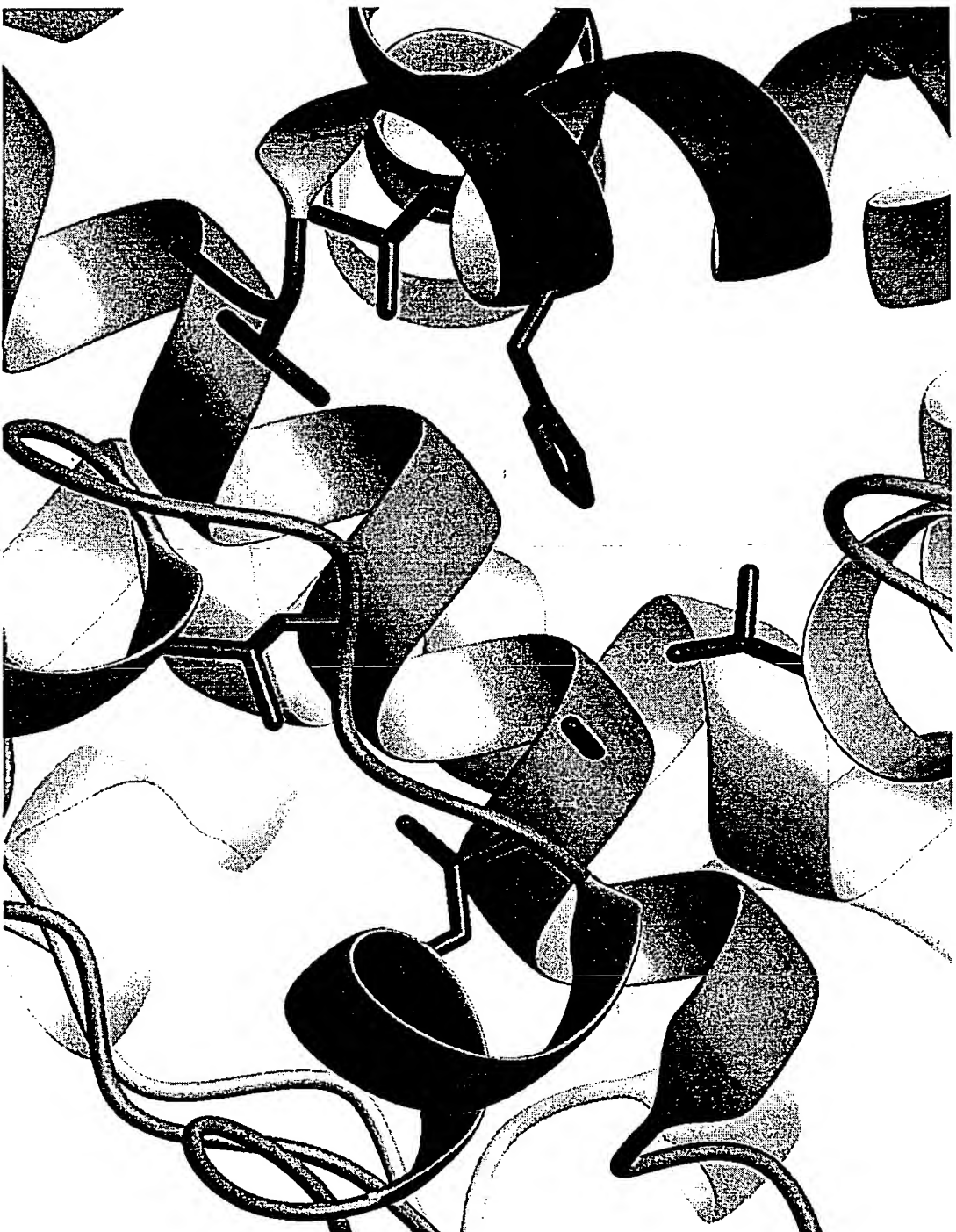


FIGURE 2C



A).                    **A-chain**                    **B-chain**  
 -----|-----  
 1 GIVEQCCTSI CSLYQLENYC NFNQFLCGS HLVEFLYLVC GERGFFYTPK T

B).                    **A-chain**                    **B-chain**  
 -----|-----  
 1 GIVEQCCTSI CSLYQLENYC NFNQFLCGS HLVEALYLVC GERGFFYTPK T

C).                    **A-chain**                    **B-chain**  
 -----|-----  
 1 GIVEQCCTSI CSLYQLENYC NFNQHL CGS HLVEFLYLVC GERGFFYTPK T

D).                    **A-chain**                    **B-chain**  
 -----|-----  
 1 GIVEQCCTSI CSLYQLENYC NFNQHL CGS HLVEWLYLVC GERGFFYTPK T

E).                    **A-chain**                    **B-chain**  
 -----|-----  
 1 GIVEQCCTSI CSLYQLENYC NFNQFLCGS HLVEWLYLVC GERGFFYTPK T

F).                    **A-chain**                    **B-chain**  
 -----|-----  
 1 GIVEQCCTSI CSLYQLENYC NFNQHL CGS HLVELLYLVC GERGFFYTPK T

G).                    **A-chain**                    **B-chain**  
 -----|-----  
 1 GIVEQCCTSI CSLYQLENYC NFNQHL CGS HLVELLYLVC GERGFFYTPK T

**Figure 4**

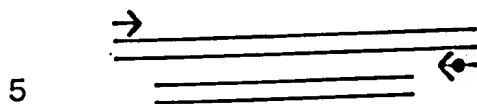
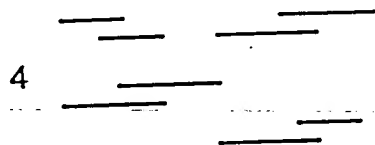
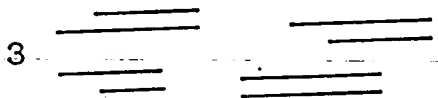
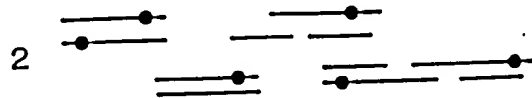
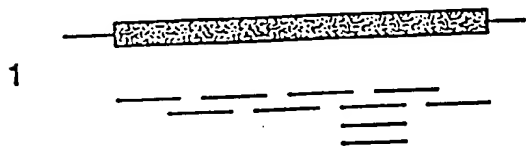
A).                      **A-chain**                      **B-chain**  
 -----|-----  
 1 NIVEQCCTSQ CSLYQYENYC NDVNQHLCGS HLVEALYLVC GERGFNYDPK T

B).                      **A-chain**                      **B-chain**  
 -----|-----  
 1 NIVEQCCTSQ CSLYQYYNFC NDKNFHLCGS HIREWLYLVC GERGFNFDPK T

C).                      **A-chain**                      **B-chain**  
 -----|-----  
 1 NIVEQCCTSQ CSLYQYYNFC NDKNFHLCLS HIREWLYLVC GERGFNFDNK T

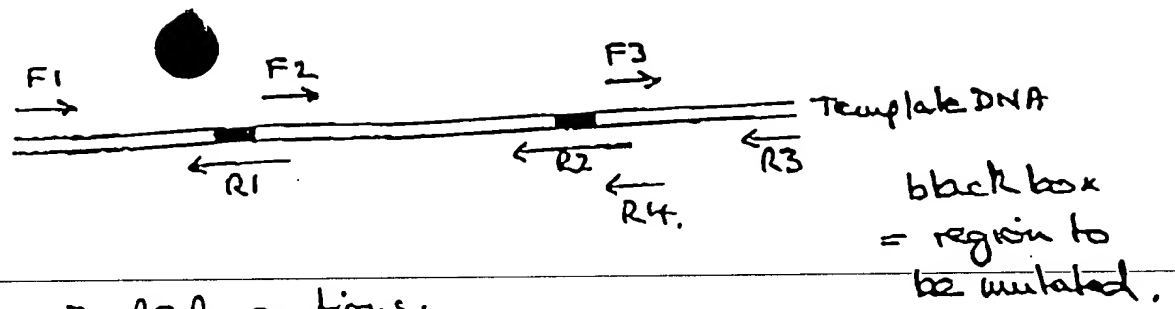
**Figure 5**





**FIGURE 6**

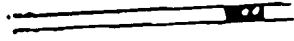




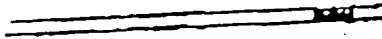
Step 1: Set up 3 PCR reactions.

Products:

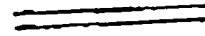
Tube 1:



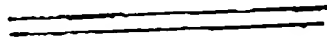
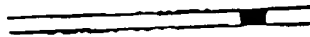
Tube 2:



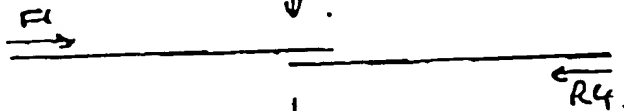
Tube 3:



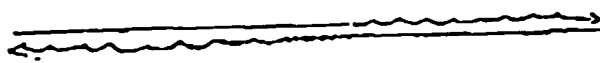
Step 2: Set up PCR reaction with products of tube 1 + products tube 2 + F1 + R4



↓ Heat + anneal phase of PCR,



↓ synthesis phase of PCR.



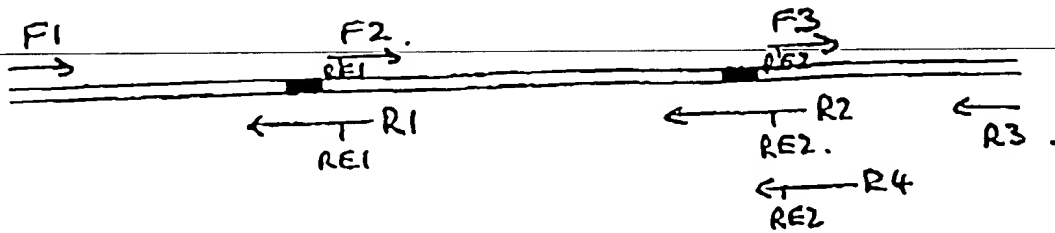
↓ amplification phase, using F1 + R4.  
during subsequent cycles.

FIGURE 8A

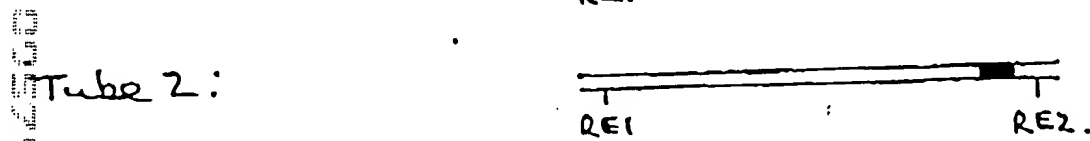
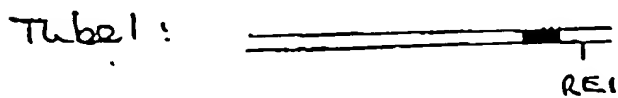
Step 3

Repeat step 2 using product from step 2 +  
product from Step 1, tube 3 + primers F1 & R3

**FIGURE 8B**



Step 1 Set up 3 PCR reactions:



Step 2: digest products from Step 1 with suitable restriction endonucleases

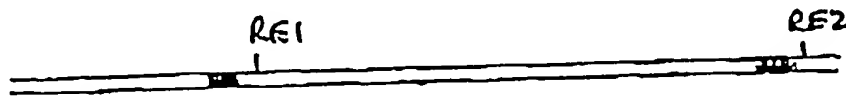
Step 3: ligate digested product from Step 2, Tube 2 with digested product from Step 2, Tube 1.



**FIGURE 9A**

Step 4

Amplify <sup>via PCR</sup> ligated products of Step 3 with F1 & R4.

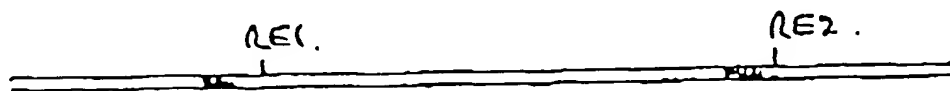


Step 5. Digest amplified product of step 4 with restriction: endonuclease #2.



Step 6.

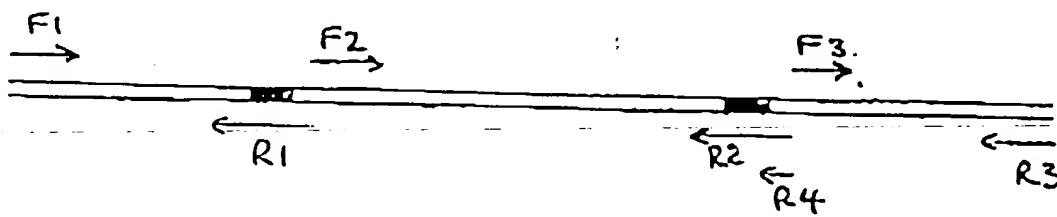
Ligate product from Step 5 with product from Step 2, tube 1.



Step 7

Amplify product from step 6 with F1 + R3

FIGURE 9B



**FIGURE 10**